Improved Attractants for Mediterranean Fruit Fly, *Ceratitis capitata* (Wiedemann): Responses of Sterile and Wild Flies to (-) Enantiomer of Ceralure B1

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J. Econ. Entomol. 96(6): 1719–1723 (2003)

ABSTRACT Tests were conducted on wild Mediterranean fruit flies, *Ceratitis capiata* (Wiedemann), in Hawaii, Italy, and Kenya, and on sterile released flies in Florida and California with a new male attractant, (−)-ceralure B1. Compared on an equal dosage basis, Mediterranean fruit fly males were significantly more attracted to the (−)-ceralure B1 than to trimedlure in each of the sites tested except for California. Compared with the standard commercial 2 g trimedlure plug, 10 mg applied on cotton wicks (Kauai) was as attractive to wild males as trimedlure after the first 2 d of the test but not after 7 d. At a dose of 40 mg (50 times less than in the 2-g plug), the (−)-ceralure B1 was significantly more attractive to male flies than the 2-g trimedlure plug for the first week of service (Florida) but not after 2 wk. Studies using released sterile flies in Florida confirm our previous work on the improved attraction of (−)-ceralure B1 (40 mg) over trimedlure. However, this trend did not hold up in a single test conducted in a residential area in California that did not show a significant difference in attraction using 20 mg of compound. Future refinements in synthesis and costs of this compound and increased availability and testing will be needed before any final evaluation in the field can be carried out.

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is a major tephritid fruit fly pest of economic importance attacking >300 different hosts, primarily temperate and subtropical fruits (Liquido et al. 1991). The threat of Mediterranean fruit fly movement and potential establishment in major U.S. fruit-producing areas currently free of Mediterranean fruit fly (i.e., California, Florida, and Texas), as well as foreign countries (e.g., New Zealand and Japan) requires that states and countries maintain a comprehensive detection program to ensure trading partners that their agricultural products are free from Mediterranean fruit fly infestation. Estimates for the impacts of established Mediterranean fruit fly populations in California alone are >$1 billion (Siebert and Cooper 1995). Countries where Mediterranean fruit fly is established must subject their host crops destined for export to costly quarantine treatments or regulated systems approaches (Jang and Moffitt 1994) to reduce the risk of entry of Mediterranean fruit fly to areas where the fly does not exist. Each year, tens of thousands of semiochemical-based traps are deployed worldwide to detect or monitor Mediterranean fruit fly populations.

Detection programs have historically been carried out using semiochemical-based lures and attractants that attract males and/or females into traps that are monitored at regular intervals. When a fly is detected, trapping is increased in and around the initial find to further delimit the extent of the population (California Department of Food and Agriculture Insect Trapping Guide 1998, FDACS-DPI 2002). The importance of early detection has always been a priority in detection, delimitation, and control programs, because costs of intervention and eventual eradication increase dramatically if the population becomes established and spreads. For the past 30 yr, *tert*-butyl 4 (or 5) chloro-2-methylcyclohexane-1-carboxylate (trimedlure) contained in Jackson traps, and McPhail traps baited with hydrolyzed protein were the primary detection tools used in Mediterranean fruit fly detection programs. Efforts to improve control and detection methods for Mediterranean fruit fly included development of both improved male lures such as ceralure (McGovern and Cunningham 1988) and more recently female-based attractants (Heath et al. 1997, Katsoyannos et al. 1999a, b).

The development of a more powerful male attractant would not only be an improvement for detection but also an effective tool for male annihilation used as part of areawide control and eradication programs. Ceralure, an iodinated analog of trimedlure was developed by McGovern and Cunningham (1988) and

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found to be more persistent than trimedlure in field trials (Avery et al. 1994; Leonhardt et al. 1996; Warthen et al. 1994, 1997). Ceralure, like trimedlure, is composed of 16 regio- and stereoisomers, of which the B1 isomer was reported to be the most attractive (Warthen et al. 1994). In 2000, a novel method for synthesis of the stereoisomers of the ceralure B1 molecule was developed and tested (Raw and Jang 2000) The (−)-enantiomer of ceralure B1 was shown to be more attractive to laboratory-released sterile flies than commercial trimedlure and commercial ceralure (Jang et al. 2001). That study also reported (−)-ceralure B1 to be more persistent than commercial trimedlure and ceralure. Until recently, no tests of the new attractant have been carried out against wild fly populations or under low populations of sterile released flies. This article follows up on the previous report (Jang et al. 2001) with results of tests of the (−)-ceralure B1 against wild Mediterranean fruit fly in Hawaii, Kenya, and Italy and sterile release flies in Florida and California where flies are being released under a preventative release program. We wanted to evaluate the new molecule against different Mediterranean fruit fly populations and to determine whether we could assess the minimum dose of (−)-ceralure B1 that would give an equivalent response to a commercial 2-g trimedlure plug as a start to developing cost-benefit analysis for the molecule. Tests were conducted in different locations to compare the attraction of the ceralure B1 molecule among several strains of Mediterranean fruit fly in Hawaii, Africa, and Italy and in Florida and California where preventative release programs are currently being carried out.

Materials and Methods

Test Compounds. Liquid trimedlure (UOP Chemicals, East Rutherford, NJ) (98% pure; density 1.02 g/ml) and trimedlure plugs and ceralure (AgriSense, Palo Alto, CA) (98% pure; density 1.43 g/ml) were purchased from commercial sources. Ceralure was stored over copper coil to prevent discoloration. The enantiomers of ceralure B1, ethyl (1*R*,2*R*,3*R*)-5-iodo-2-methylcyclohexane-1-carboxylate and ethyl (1*S*,2*S*,3*S*)-5-iodo-2-methylcyclohexane-1-carboxylate (98% pure), referred to as (−)-ceralure B1 and (+)-ceralure B1, respectively, based on their optical rotations, were synthesized by using a unique nine-step process (Raw and Jang 2000). Racemic ceralure was synthesized in the same way with the exception of using racemic siglure acid as starting material. Chemicals were compared based on weight and not corrected for volatility. All treatment dosages were formulated in acetone and presented in 100-μl aliquots on a cotton wick.

Wild Fly Field Test: Hawaii. Responses of wild fly populations to the various semiochemical male attractants were conducted in commercial coffee fields on the island of Kauai, HI. Chemical treatments were applied to a 1.2 by 0.7-cm (diameter) cotton wick, using an Eppendorf pipetter and placed in a Jackson trap with a removable sticky insert. Aliquots (100 μl) containing 10 mg of each treatment chemical in acetone were tested. Treatments were as follows: trimedlure, ceralure, (−)-ceralure B1, (+)-ceralure B1, (±)-racemic ceralure, and control (acetone). Traps were located in a commercial coffee orchard (a preferred Mediterranean fruit fly host) with trees spaced 3 m between rows. Traps were placed ≈1 m from the ground in individual trees in every other row and ≈5 m apart within a row (every 10 trees). Traps were deployed using a randomized complete block design and serviced 48 h after being placed in the field. There were 50 replicates (traps) per treatment over five different weeks (10 of each treatment per week).

A second test was conducted with wild flies comparing 10 mg of racemic ceralure, (−)-ceralure B1, and (+)-ceralure B1 against a 2-g trimedlure polymer plug (AgriSense), the standard male lure used in detection programs. These tests were run to determine the minimum dose of the (−)-ceralure B1 needed to compete with the standard 2-g plug. Treatments were tested on cotton wicks in Jackson traps. Traps were placed in a randomized complete block design in coffee fields as described above. Traps were serviced 2 and 7 d after placement in the field. There were 40 replicates (traps) per treatment over four different weeks (10 of each treatment per week).

Wild Fly Field Tests: Kenya and Italy. To compare responses of wild flies at different locations, tests were also conducted in Kenya, Africa, and in Rome, Italy. For the African tests, 20 mg of (−)-ceralure B1 was compared with 20 mg of trimedlure. Compounds were placed on a 1.2 by 0.7-cm cotton wick in a Jackson trap. Traps were placed in a commercial coffee field with trees spaced 0.5 m apart and 1.5 m between rows. Traps were placed 1 m from the ground in every fifth tree and every other row (spacing of ≈2.5–3 m). Traps were serviced after 48 h and five replicates (traps) per treatment were placed in the field in one test.

Similar tests were also conducted in Italy except that traps were hung in a commercial citrus orchard with trees spaced 4 m apart. Jackson traps were placed 1.5 m off the ground in every third tree in every third row. Ten milligrams of (−)-ceralure B1 on a 1.2 by 0.7-cm cotton wick was compared with a 2-g trimedlure plug. Traps were serviced at 7 d and consisted of 25 replicates (traps) per treatment over five different weeks (five of each treatment per week).

Sterile Fly Response Tests: Florida and California. Additional tests of the compounds were made using aerial released adult sterile Mediterranean fruit fly in experiments in Tampa, FL. Tests were conducted to evaluate the response of flies to lures under lower populations where no established populations of Mediterranean fruit fly exist. The released flies were part of the USDA/Florida Medfly Preventative Release Program in the Tampa area where 125,000 sterile adult male flies are released weekly per square mile via air. The sterile Mediterranean fruit fly strain used in these tests were the Toliman Vienna eight male only genetic sexing strain developed by and obtained from the USDA–APHIS mass-rearing facility in El Pino, Guatemala. Compounds were tested on 1.2 by 0.7-cm cotton wicks in Jackson traps. The treatments were 40 mg of (−)-ceralure, 40 mg of trimedlure, and control. Traps were randomly placed in a citrus orchard (≈15 by 12 m between traps) using eight replicates (traps) of each treatment and four control traps. Traps were inspected weekly for 4 wk. Data were not transformed before analysis based on even distribution (releases) of the sterile flies by aircraft using global positioning system-mapped flight lines within the release area.

A second series of tests in Florida compared 40 mg of (−)-ceralure B1 with 40 mg of trimedlure and a commercial 2-g TML plug (AgriSense). The experimental design of the second test was identical to the previous Florida test (described above) with the exception of having 15 replicates (traps) of each treatment and five control traps and checking of the traps weekly for 6 wk instead of 4 wk.

A test was also conducted in California with aerial released sterile Mediterranean fruit fly as part of the USDA–APHIS, California Department of Food and Agriculture California Medfly Preventive Release Program. The flies (Toliman Vienna eight all male genetic sexing strain) were obtained from the USDA–APHIS Mediterranean fruit fly mass-rearing facility in El Pino, Guatemala, as in the Florida test. Twenty milligrams of (−)-ceralure B1 was compared with 20 mg of trimedlure and control. Treatments were placed on a 1.2 by 0.7-cm cotton wick and placed in Jackson traps with removable sticky inserts. Traps (one of each treatment) were placed in 11 different sites in and around the residential section of East Los Angeles. Traps were randomly placed in a 7.77-km^2 radius in backyards or commercial properties having host trees, primarily citrus. Where no host trees were available, a nonhost tree was used. Traps were serviced after 7 d.

Data Analysis. Data contain male trap captures only because ceralure and TML attract primarily male flies. Females captured were not included in the data. Trap captures of wild flies in Hawaii, Kenya, and Italy were presented as the mean number of flies captured per trap per day. A square root (x + 0.5) transformation was performed on the data; however untransformed means are presented in the tables. Data of sterile released flies in California and Florida were presented as mean values ± SEM and not transformed. An analysis of variance (ANOVA) (PROC GLM) was performed on all the data followed by Tukey's test for mean separation. Significant differences were determined at the $P < 0.05$ level. Statistical analysis was run on SAS version 8.2 (SAS Institute 1990).

Results

In initial wild fly studies in Kauai, comparing 10 mg of ceralure compounds with 10 mg of TML, (−)-ceralure B1 had a significantly higher trap capture (flies per trap per day) compared with racemic ceralure, which was significantly different than (+)-ceralure B1 and commercial ceralure, which was significantly different from TML, which was significantly different from control. (Table 1a) ($F = 55.98$, $P < 0.0001$). In the second Hawaii experiment comparing

Table 1a. Trap capture of wild Mediterranean fruit flies to 10-mg doses of male attractants in open field tests on Kauai

Treatment	Male fly capture (mean flies/trap/d ± SEM)	
	N	
Trimedlure (10 mg)	50	7.05 ± 1.1d
Commercial ceralure (10 mg)	50	15.3 ± 2.09c
(+)-Ceralure B1 (10 mg)	50	20.2 ± 4.1c
(±)-Ceralure B1 (10 mg)	50	35.4 ± 5b
(−)-Ceralure B1 (10 mg)	50	52.1 ± 6.8a
Acetone control	50	0.24 ± 0.06e

Data analyzed by Proc GLM; means followed by same letter in a column are not significantly different ($P < 0.05$) by Tukey's test.

Table 1b. Trap capture of wild Mediterranean fruit flies to 10-mg ceralure compounds versus trimedlure plug in open-field tests on Kauai

Treatment	Male fly capture (mean flies/trap/d ± SEM)		
	N	2-d aged	7-d aged
Trimedlure 2-g plug	40	39.4 ± 8.7ab	29.8 ± 4a
(+)-Ceralure B1 (10 mg)	40	17.7 ± 2.9b	2.9 ± 0.97c
(±)-Ceralure B1 (10 mg)	40	41.9 ± 8.7a	7.5 ± 1.6b
(−)-Ceralure B1 (10 mg)	40	58.5 ± 11.2a	10.1 ± 2.1b
Acetone control	40	0.26 ± 0.07c	0.03 ± 0.01d

Data analyzed by Proc GLM; means followed by same letter in a column are not significantly different ($P < 0.05$) by Tukey's test.

10 mg of ceralure with the commercial 2-g TML plug (a difference of 200 times less material), the results after 2 d showed that (−)-ceralure B1 was not significantly different from racemic ceralure or the trimedlure plug. The results after 7 d showed that TML plug had the highest trap capture and results were significantly different from the other treatments (Table 1b) ($F = 25.91$, $F = 46.97$, $P < 0.0001$).

The results of tests that were conducted with wild populations in Kenya showed that 20 mg of (−)-ceralure B1 captured significantly more flies than 20 mg of trimedlure (Table 2a) ($F = 35.86$, $P = 0.0003$). In open field tests in Italy after 7 d, there was no significant difference in trap capture between 10 mg of (−)-ceralure B1 and 2 g of trimedlure (plug). (Table 2b) ($F = 0.37$, $P = 0.5451$).

The responses of aerial released sterile Mediterranean fruit fly in Florida were similar to responses of wild Mediterranean fruit fly. There were significantly more males captured in traps containing (−)-ceralure B1 compared with trimedlure and control for 3 of 4 wk (Table 3a) ($F = 53.89$, $P < 0.0001$ for week 1; $F = 44.03$, $P < 0.0001$ for week 2; $F = 5.84$, $P = 0.0117$ for week 3; and $F = 4.54$, $P = 0.0263$ for week 4) and not significantly different at week 4. In a second test (Table 3b), 40 mg of (−)-ceralure B1 captured significantly more males than 40 mg of trimedlure, the trimedlure plug, or control after the first week service ($F = 11.47$, $P < 0.0001$) and still caught flies in weeks 2–5, whereas an equivalent dose of trimedlure did not catch any flies after week 1. In week 2, 40 mg of (−)-ceralure B1 was not significantly different from

Table 2a. Trap captures of wild Mediterranean fruit flies in open field tests in Kenya

Treatment	N	Mean ± SEM (flies/trap/d)
(−)-Ceralure B1 (20 mg)	5	102.5 ± 4.7a
Trimedlure (20 mg)	5	40.3 ± 8b

Data analyzed by Proc GLM; means followed by same letter in a column are not significantly different ($P < 0.05$) by Tukey's test.

Table 2b. Trap captures of wild Mediterranean fruit flies in open field tests in Italy

Treatment	N	Mean ± SEM (flies/trap/d)
(−)-Ceralure B1 (10 mg)	25	8.4 ± 2.1a
Trimedlure (2-g plug)	25	6.3 ± 1.5a

Data analyzed by Proc GLM; means followed by same letter in a column are not significantly different ($P < 0.05$) by Tukey's test.

the 2-g trimedlure plug, but they were both significantly different from 40 mg of trimedlure and control ($F = 27.08$, $P < 0.0001$). In weeks 3, 4, 5, and 6, the 2-g trimedlure plug captured significantly more males than any of the other treatments (Table 3b) ($F = 33.78$, $F = 41.65$, $F = 51.42$, and $F = 21.47$; $P < 0.0001$).

Tests in California were carried out in an urban residential area under the preventative release program rather than in agricultural areas. The results of aerial released sterile medflies in California showed that 20 mg of (−)-ceralure B1 did not capture more male Mediterranean fruit fly than 20 mg of trimedlure (Table 4) ($F = 6.96$, $P = 0.0033$).

Discussion

Wild male Mediterranean fruit fly in Kauai and Kenya showed a greater response to (−)-ceralure B1 than to commercial ceralure (a mixture of 16 isomers and enantiomers), or trimedlure when compared at equal doses. This confirms our previous studies with released sterile Mediterranean fruit flies in Hawaii (Jang et al. 2001), showing the superior attraction of this molecule (up to 9 times more attractive) than trimedlure, especially the (−)-configuration. One exception was the single test conducted in California within the preventative release program grid in a residential area of Los Angeles. We speculate that perhaps our results might be due to the fact that within the grid, availability of host trees are limited and may have influenced the relative populations in the area. Previous studies by Warthen et al. (1994) and by Leonhardt et al. (1996) had reported that the (±)-B1 isomer was more attractive than the other *trans*- or *cis*-isomers of ceralure, which had little or no attraction. The (±)-ceralure B1 isomer was reported by Leonhardt et al. (1996) to be 2–3 times as active as isomer C of trimedlure (the most attractive isomer of trimedlure), and pure (−)-ceralure B1 is even more attractive (E.B.J., unpublished observations). Thus, the (−) ceralure B1 seems to be one of the most attractive male lures yet evaluated against Mediterranean fruit fly.

We also wanted to compare the (−)-ceralure B1 to the commercial 2-g trimedlure polymer plug now in use in thousands of detection traps worldwide to find out whether these relatively low doses (10–40 mg) could compete with the 2-g plug. These studies generally showed an equal or better attraction of flies for the first day-week of the test, after which the plug outperformed the 10–40 mg on a cotton wick. We attribute this to the much greater load of material in the trimedlure plugs (2,000 mg), which have been formulated to last 4–6 wk in the field. It is obvious from these initial tests that amounts greater than ≈50 mg will likely be needed to effectively compete with the commercial trimedlure plugs. However, this is still ≈40 times less material than in a 2-g plug of trimedlure.

Due to the limited availability of enantiomerically pure synthesized (− and/or +)-ceralure B1, the test conducted in these studies varied in their applied doses as well as comparisons on longevity. Future tests will look at whether pure (99%) (−)-ceralure B1 is in

Table 3a. Response of released (sterile) Mediterranean fruit flies in open field tests in Tampa, FL

Treatment	N	Mean fly capture ± SEM			
		Week 1	Week 2	Week 3	Week 4
(−)-Ceralure B1 (40 mg)	8	50 ± 5.2a	7.6 ± 0.98a	4.1 ± 1.4a	0.87 ± 0.35a
Trimedlure (40 mg)	8	6.4 ± 1.3b	0 ± 0b	0 ± 0b	0 ± 0a
Control	4	0.25 ± 0.25b	0 ± 0b	0 ± 0b	0 ± 0a

Data analyzed by Proc GLM; means followed by same letter in a column are not significantly different ($P < 0.05$) by Tukey's test.

Table 3b. Comparison of trap captures between ceralure B1, liquid trimedlure and the polymer trimedlure plug against released (sterile) Mediterranean fruit fly in Tampa, FL

Treatment	N	Mean fly capture ± SEM					
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
(−)-Ceralure B1 (40 mg)	15	8.5 ± 0.99a	5.3 ± 0.74a	1.5 ± 0.45b	0.42 ± 0.17b	0.07 ± 0.07b	0 ± 0b
Trimedlure (40 mg)	15	2.2 ± 0.54b	0 ± 0b	0 ± 0b	0 ± 0b	0 ± 0b	0 ± 0b
Trimedlure 2-g plug	15	4.6 ± 0.66b	8.2 ± 0.94a	21.8 ± 3a	7.8 ± 0.98a	7.8 ± 0.93a	5.1 ± 1a
Control	5	2.8 ± 1.9b	1.6 ± 0.67b	2.4 ± 1.4b	0.8 ± 0.6b	0 ± 0b	0.2 ± 0.2b

Data analyzed by Proc GLM; means followed by same letter in a column are not significantly different ($P < 0.05$) by Tukey's test.

Table 4. Response of released (sterile) Mediterranean fruit flies in open field tests in California

Treatment	*N*	Mean capture ± SEM (flies/trap/d)
(−)-Ceralure B1 (20 mg)	11	109 ± 33a
Trimedlure (20 mg)	11	48 ± 12ab
Control	11	0.27 ± 0.27b

Data analyzed by Proc GLM; means followed by same letter in a column are not significantly different ($P < 0.05$) by Tukey's test.

fact necessary for a commercial product or whether newer synthesis routes (Khrimian et al. 2003) might reduce the costs of manufacturing the racemic ceralure B1. Racemic ceralure B1 has shown itself to be nearly as attractive to Mediterranean fruit flies as the (−)-ceralure B1 and significantly better that TML on a weight-to-weight basis (Table 1a). Even if the racemic ceralure B1 is not more cost effective to produce, rather than abandoning ceralure B1 due to cost, perhaps its use in a delimiting survey mode after the detection of Mediterranean fruit fly using trimedlure, may justify its expense. The increased costs of ceralure B1 might also be justified by its use in high-risk (port and airport) areas.

Ceratitis capitata is a worldwide pest of agriculture and thus the need to continually improve methods for detection and areawide control of this pest is of world importance. Our studies have shown that Mediterranean fruit fly response to (−)-ceralure B1 is comparable in several different locations and thus holds promise that, if it can be produced cost effectively, could replace or supplement trimedlure as the standard survey lures for male Mediterranean fruit fly detection and delimitation. The increased attraction of (−)-ceralure B1 compared with trimedlure will greatly improve efficacy in areawide control and detection and delimiting programs. This increase in attraction by this compound will also improve the potential to use mass trapping to achieve male annihilation, a method used successfully to control oriental fruit fly, *Bactrocera dorsalis* Hendel using methyl eugenol. Further testing will be required to determine whether its use in male annihilation of Mediterranean fruit fly is possible.

Acknowledgments

We thank Charlie Rodd (USDA–ARS–PBARC, Kapaa, Kauai) for assistance in field trapping studies. We also thank Robert Dowell and Dick Penrose (California Department of Food and Agriculture) for help in trapping evaluations in Los Angeles area, Carlo Tronci and Vincenzo Di Ilio (BBCA, Italy) for help in trapping studies conducted in Italy, and Mark Salvato (USDA–APHIS, Florida) for assistance.

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Received for publication 3 June 2003; accepted 24 August 2003.